NO:2.

3. (Twice Amended) A crystallized complex for use in X-ray crystallography comprising:

a. an HCV NS3 helicase protein selected from SEQ ID NO:2; fragments of SEQ ID NO:2 comprising at least amino acids 183 to 582; mutants of SEQ ID NO:2 containing one or more of the following amino acid substitutions: Ser231-to-Ala, Thr269-to-Ala, Ser370-to-Ala, Thr411-to-Ala, Trp501-to-Phe, Trp501-to-Leu or Trp501-to-Ala, Gln460-to-Ala, Arg461-to-Ala, Arg462-to-Ala, Arg464-to-Ala, or Arg467-to-Ala; or fragments of SEQ ID NO:2 comprising at least amino acids 183 to 582 and containing one or more of the following amino acid substitutions: Ser231-to-Ala, Thr269-to-Ala, Ser370-to-Ala, Thr411-to-Ala, Trp501-to-Phe, Trp501-to-Leu or Trp501-to-Ala, Gln460-to-Ala, Arg461-to-Ala, Arg462-to-Ala, Arg464-to-Ala, or Arg467-to-Ala; and

C1

b. a single-stranded oligonucleotide consisting of between 6 and 12 nucleotides.

REMARKS

Applicants have cancelled claim 4 without prejudice to their right to file for and obtain claims directed to the canceled subject matter in applications claiming priority from the present application under 35 U.S.C. §120.

Applicants have amended claims 1-3 in response to the Examiner's

rejections. In particular, applicants have amended claims 1 to 3 to clarify that the claimed crystallizable compositions comprise a single-stranded oligonucleotide of between 6 and 12 nucleotides.

Claims 1-4 stand rejected under 35 U.S.C. §112, first paragraph as lacking enablement for compositions comprising oligonucleotides other than dU_8 and oligonucleotides "highly related" to dU_8. According to the Examiner, Brown et al., "makes clear that crystallization of protein-DNA complexes is not predictable and requires guidance and extensive experimentation that would not be considered routine to develop crystals suitable for X-ray crystallography." Applicants traverse.

A close reading of Brown et al., demonstrates that the criticality of choice of oligonucleotides for co-crystallization with a protein correlates with the role of the oligonucleotide in crystal lattice formation. When oligonucleotides do not play a role and therefore the choice of sequence of the oligonucleotide is much less important and is determined by the ability to bind to the protein.

> "In contrast, the co-crystal structures of Klenow and Dnase I show the DNA to be covered mostly by protein, where protein-protein interactions form the majority of crystal lattice contacts. In these specific cases, the choice of oligomers was based on the previously determined three-dimensional structures of the proteins....
>
> DNA stacking in some cases appears to be important, and , therefore the terminal base sequences should be varied. However, depending upon the architecture of the protein, the DNA can be completely enveloped, and would therefore have little effect on cocrystallizations." (emphasis added) (Brown et al., p. 302, 2nd and 3rd paragraphs).

As set forth in the application, the crystal structure of HCV NS3 helicase complexed with dU_8 clearly demonstrates that the oligonucleotide does not play a significant role in crystal lattice formation:

"[T]he oligonucleotide is most tightly bound at the 3' and 5' ends with few contacts [between the protein and the oligonucleotide] with the central nucleotides." (emphasis added) (p. 70, lines 4-7).

* * * *

"Sequence specific interactions with the DNA bases are not observed within the central binding cavity of the helicase." (p. 71, lines 1-2).

Because the oligonucleotide does not play a role in the HCV NS3 helicase-oligonucleotide complex crystal lattice formation, the affinity of the oligonucleotide for the protein will determine whether a crystal complex can be formed. Prior to the effective filing date of this application it was known that HCV NS3 helicase was capable of binding all of the different homopolymeric polynucleotides. For example, Y. Gwack et al., "Characterization of RNA Binding Activity and RNA Helicase Activity of the Hepatitis C Virus NS3 Protein," Biochem. Biophys. Res. Comm., 225, pp. 654-69 (1996) ("Gwack"; copy enclosed) demonstrated that poly(A), poly(U), poly(G) and poly(C) each bound to HCV NS3 helicase (p. 656, Fig. 2D and p. 659, first paragraph), albeit with different affinities.

The fact that oligonucleotides complexed with HCV NS3 helicase do not play a role in crystal lattice formation, coupled with the demonstration that each of poly(A), poly(U), poly(G) and poly(C) can bind to the helicase, supports the full scope of amended claim 1 and its dependent claims. One of skill in the art would reasonably expect all oligonucleotides within the scope recited in claim 1 (i.e., any single stranded nucleotide consisting of between 6 to 12 bases) to bind to HCV NS3 helicase and form a crystallizable complex with that protein. Accordingly, amended claims 1 to 3 are fully enabled. In light of this, applicants request that the Examiner reconsider and drop her

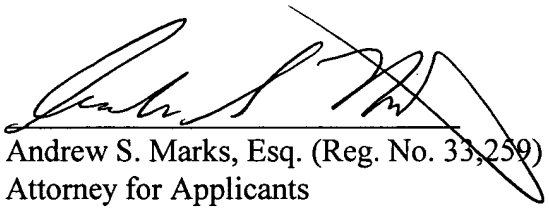
rejection under 35 U.S.C. 112, first paragraph.

Claims 2-4 stand rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter applicants regard as their invention. Specifically, the Examiner objects to the recitation of "nucleotide pairs" in claims 2 and 3 because "the specification makes cleat that only single-stranded oligonucleotides are crystallized, not double stranded oligonucleotides." The Examiner also notes that if claim 3 is amended to delete the recitation "nucleotide pairs", then claim 4 would be duplicative of claim 3. Applicants have obviated this rejection by deleting the term "nucleotide pairs" from amended claims 2 and 3 (as well as from claim 1) and by canceling claim 4.

Applicants stress that these amendments are made simply to clarify that the complexes formed in the crystallizable compositions of this invention are between NS3 helicase and a single-stranded oligonucleotide. The application makes clear that the claimed compositions may initially comprised a double-stranded oligonucleotide that is subsequently dissociated into single stranded oligonucleotides prior to crystallization (p. 14, lines 5-8). The claim amendment does not exclude from the scope of the claim compositions that initially contain double-stranded oligonucleotides and are subsequently dissociated into single-stranded nucleotides. This is because the metes and bounds of the claim are met once dissociation has occurred.

Applicants request that the Examiner consider the foregoing remarks, enter the indicated amendments and allow the claims pending in this application to pass to issue.

Respectfully submitted,



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APPENDIX 1

1. (Twice Amended) A crystallizable composition capable of producing crystals for use in X-ray crystallography comprising:

a. an HCV NS3 helicase protein selected from SEQ ID NO:2; fragments of SEQ ID NO:2 comprising at least amino acids 183 to 582; mutants of SEQ ID NO:2 containing one or more of the following amino acid substitutions: Ser231-to-Ala, Thr269-to-Ala, Ser370-to-Ala, Thr411-to-Ala, Trp501-to-Phe, Trp501-to-Leu or Trp501-to-Ala, Gln460-to-Ala, Arg461-to-Ala, Arg462-to-Ala, Arg464-to-Ala, or Arg467-to-Ala; or fragments of SEQ ID NO:2 comprising at least amino acids 183 to 582 and containing one or more of the following amino acid substitutions: Ser231-to-Ala, Thr269-to-Ala, Ser370-to-Ala, Thr411-to-Ala, Trp501-to-Phe, Trp501-to-Leu or Trp501-to-Ala, Gln460-to-Ala, Arg461-to-Ala, Arg462-to-Ala, Arg464-to-Ala, or Arg467-to-Ala; and

b. [an] a single stranded oligonucleotide consisting of between 6 and 12 nucleotide [or nucleotide pairs].

2. (Twice Amended) The composition according to claim 1, wherein said HCV NS3 helicase protein comprises amino acids 167-631 of SEQ ID NO:2 [and wherein said oligonucleotide is a single stranded polynucleotide consisting of between 6 and 12 nucleotides or nucleotide pairs].

3. (Twice Amended) A crystallized complex for use in X-ray

crystallography comprising:

a. an HCV NS3 helicase protein selected from SEQ ID NO:2; fragments of SEQ ID NO:2 comprising at least amino acids 183 to 582; mutants of SEQ ID NO:2 containing one or more of the following amino acid substitutions: Ser231-to-Ala, Thr269-to-Ala, Ser370-to-Ala, Thr411-to-Ala, Trp501-to-Phe, Trp501-to-Leu or Trp501-to-Ala, Gln460-to-Ala, Arg461-to-Ala, Arg462-to-Ala, Arg464-to-Ala, or Arg467-to-Ala; or fragments of SEQ ID NO:2 comprising at least amino acids 183 to 582 and containing one or more of the following amino acid substitutions: Ser231-to-Ala, Thr269-to-Ala, Ser370-to-Ala, Thr411-to-Ala, Trp501-to-Phe, Trp501-to-Leu or Trp501-to-Ala, Gln460-to-Ala, Arg461-to-Ala, Arg462-to-Ala, Arg464-to-Ala, or Arg467-to-Ala; and

b. a single-stranded oligonucleotide consisting of between 6 and 12 nucleotides [or nucleotide pairs].

Claim 4 has been canceled.